Fascicular Sutureless and Suture Repair of the Peripheral Nerves

A Comparison Study in Laboratory Animals

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Peripheral nerve repair remains one of the most frustrating problems in hand surgery. It is well known that the results of conventional epineurial nerve repair are unpredictable and often inadequate.[1-5] Recovery of normal function for adult patients requiring finely controlled movements of the fingers for their occupations rarely occurs;[4] and in median nerve repairs, no more than 50% of the patients will obtain a functional recovery better than protective sensibility.[5]

Conventional nerve repairs use sutures to approximate the outermost connective tissue layer, the epineurium. With this technique the nerve may externally appear to be adequately repaired, but a number of studies have reported the low percentage of satisfactory approximations of the fascicular bundles within the epineurium.[6-8] They have pointed to overriding, gapping, buckling, and straddling of these components as the cause. Hence, an operative technique that results in a more normal anatomical configuration of the fascicular bundles at the anastomosis might be expected to produce better results from conventional epineurial techniques. The development of microsurgical techniques made fascicular perineurial nerve repair a feasible alternative to epineurial nerve repair. In perineurial nerve repair, the individual fascicles or groups of fascicles are approximated by suturing the perineurium.

Sunderland states that there is no controversy between epineurial and perineurial repair, only specific indications for each type of repair.[9] In spite of Sunderland's arguments, there still exists an active controversy as to whether fascicular repair results in a better functional recovery than epineurial nerve repair. Experimental studies have been unable to resolve this controversy.[10-16] Methodologic problems with both the experimental models and the

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evaluation methods may have led to inconclusive results in many of these studies. Clinical studies have reported perineurial suture repair to result in improved functional results compared to epineurial repair.[17-20]

The results of clinical nerve repair by epineurial or perineurial suture repair remain a major limitation to rehabilitation of the patient. An alternative solution to improve the functional results of suture repair may be a sutureless technique of nerve repair. Sutureless tubulization techniques of nerve repair hold the two ends of the severed nerve in close approximation either by wrapping membrane around the site of discontinuity or by passing a preformed tube over the two ends of the nerve (Fig 1).

Numerous sutureless techniques of nerve repair have been investigated over the past 100 years.[21-22] Past tubulization techniques have not been widely accepted clinically due to excessive tissue reactions from many of the tubulization materials[21-26] and, more recently, to the failure of *epineurial* tubulization with less reactive materials to show a statistically significant improvement in function compared to suture methods.[26-30] It may be possible that the failure of epineurial tubulization is a function of the application or evaluation methods used in these studies.

The aim of this study was to evaluate a *fascicular* technique of sutureless nerve repair with hypoantigenic collagen membrane. In contrast to past epineurial tubulization techniques this technique wraps the membrane around the perineurium. Using quantitative physiological methods, we compared tubulization, suture, and fibrin blood clot repairs at the fascicular level.

Methods and Materials

Thirty Sprague-Dawley white male rats weighing 250 gm each were studied. Twenty animals were used to compare nerve repairs and 10 animals were used as controls.

Experimental Model

Adult rats were anesthetized with intraperitoneal sodium pentobarbital. The saphenous nerve in the rat was used in this study. The saphenous nerve is usually a single major fascicle (0.3–0.5 mm diameter), although occasionally there are one or two additional smaller fascicles. It can be transected and repaired without tension. Bilateral transection causes minimal

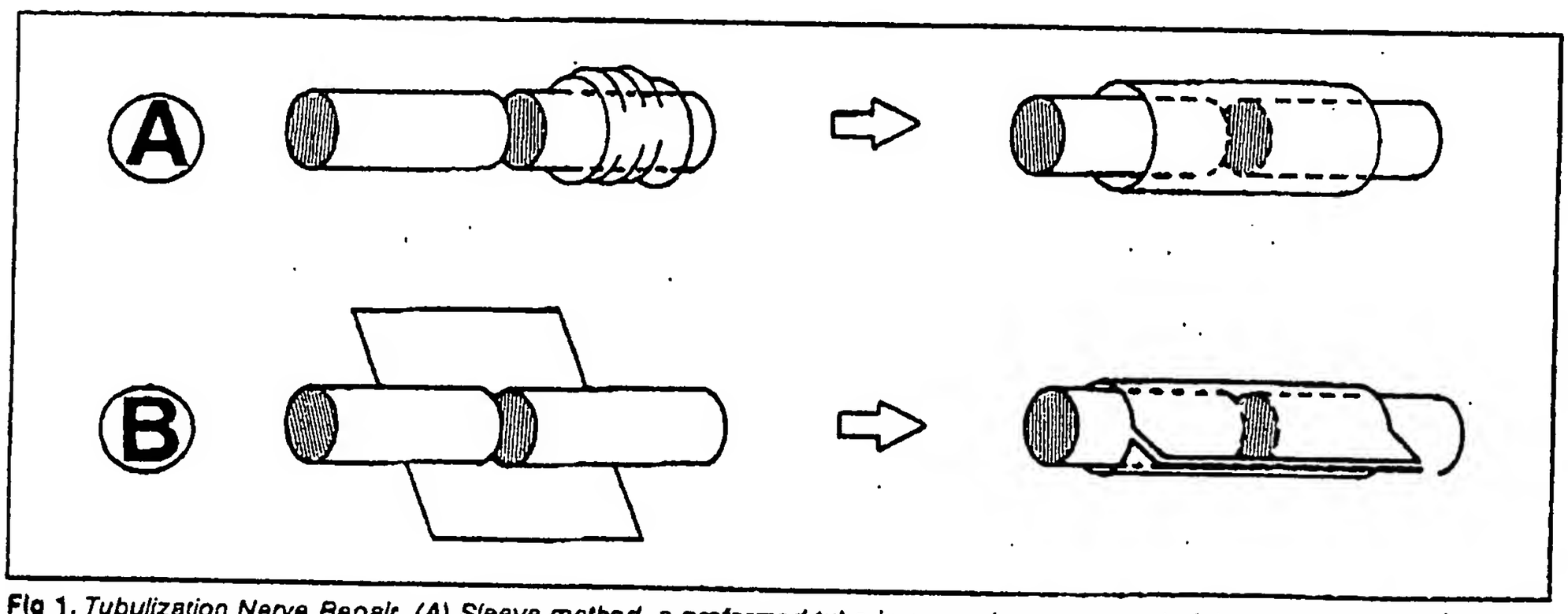

Fig 1. *Tubulization Nerve Repair. (A) Sleeve method, a preformed tube is passed over one end of the nerve (above left) and is then pulled over the anastomosis (above right). (B) Single leaf method, membrane is passed under nerve (below left) and then wrapped around nerve to form a tube (below right).*

functional deficits in rats. All surgical dissection and repair was done under magnification (16–25×).

Nerve Repairs

Both saphenous nerves were exposed 10 mm above the knee by a transverse incision. Five millimeters of epineurial connective tissue was removed on either side of the planned transection site. In the repair by tubulization, a 5 × 5 mm hypoantigenic collagen membrane* (0.07 mm in thickness) was slipped under the intact nerve, and the nerve was severed with a microscissor. The two sides of the membrane were brought together around the nerve (Fig 1B). The tube was then sealed by applying pressure with a serrated clamp to the two apposed sides of the membrane. The enclosing membrane and the formation of a fibrin blood clot hold the nerve ends together. In the repair by perineurial suture, a 5 × 5 mm sheet of rubber was passed under the nerve as a platform, and the nerve was severed as in the tubulization repair.

One to three 10-0 nylon sutures with ST-7 needles (Ethicon) were placed in the perineurium. The sutures and the formation of a fibrin blood clot hold the nerve ends together. In the repair by fibrin blood clot, the nerve was transected and an autologous fibrin blood clot was used to hold the ends of the nerve in apposition. One 10-0 nylon suture was placed in the surrounding connective tissue to mark the site of repair. No postoperative immobilization was used in any of the above repairs.

Controls

Uninjured and crushed nerves served as controls. The uninjured nerves were not surgically dissected. The crushed nerves were surgically dissected in the same manner as the transected (repaired) nerves. The nerve was crushed with a jeweler's forceps for 15 seconds over a 1 mm section. This is sufficient to cause complete Wallerian degeneration (as verified histologically in three animals not included in this study).

Physiological Evaluation

Evaluation was done by physiological determination of the area of the CAP (compound action potential). The measurement of the area under the CAP for the quantitative evaluation of nerve regeneration by means of the IMCAP (Integrated Monophasic Compund Action Potential) has been recently described by the authors.[33] The IMCAP measures the physiological activity of the entire myelinated axonal population, in contrast to the peak height of the CAP which measures a subpopulation of the faster myelinated axons and the conduction velocity which measures the fastest fibers that contribute to the CAP.[34]

For physiological evaluation, the nerves from the inguinal ligament to the ankle were removed from the anesthetized animal by careful dissection under a binocular microscope at 25× power. The nerves were placed in a recording chamber (Fig 2) on a linear array of Ag-AgCl electrodes. The conduction distances were as follows: proximal stimulator to recording electrode was 15 mm, and distal stimulator to recording electrode was 25 mm. The repair site was positioned between the proximal and distal stimulating electrodes. In the case of the control nerves this "repair" corresponds with either a zone of no injury or a crush injury.

The nerve was kept under Tyrode's solution at 35°C, through which bubbled 95% O_2 with 5% CO_2. For recording purposes the nerve on the electrodes was raised into mineral oil that is floating on the Tyrode's solution. The proximal end of the nerve was crushed to obtain monophasic recordings of the compound action potential. Amplification was by a DATA, Inc, Model 2124 with a bandpass of 0.05 Hz–10 kHz. The voltage from the amplifier was led to a

*The collagen membrane used in this study was developed by the Collagen Corporation of Palo Alto, California.[31,32]

Princeton Applied Research CW-1 Boxcar Integrator specially modified for purely linear integration. The integrator was gated on and off by internal circuitry synchronized to our stimulus. The output of the CW-1 is proportional to the integrated area and is read directly on a 3½ digit digital voltmeter. The CW-1 is a highly accurate instrument, which we keep in thermal equilibrium, and thus can achieve a replicability of a fixed area input within 1%.

Biological variability exceeded that of the instrumentation. To reduce this source, we summed each response 30 times. The summed area shows a replicability from run-to-run of 98%. This accuracy is sufficient for the results which we report.

Stimuli were generated by a Grass S8 stimulator with two Model 478A stimulus isolation units. Stimulus strengths were 50% above the maximal needed to stimulate the total population of myelinated axons as measured by the IMCAP. The response, the time of stimulus, the time of the integrated gate, and time marks from a crystal-controlled generator were photographed on polaroid film from a Tektronix 565 oscilloscope (Fig 2).

The IMCAP recorded from stimulation of the *distal* electrode is a measure of those *proximal* myelinated axons at the recording electrode that connect across the repair site.

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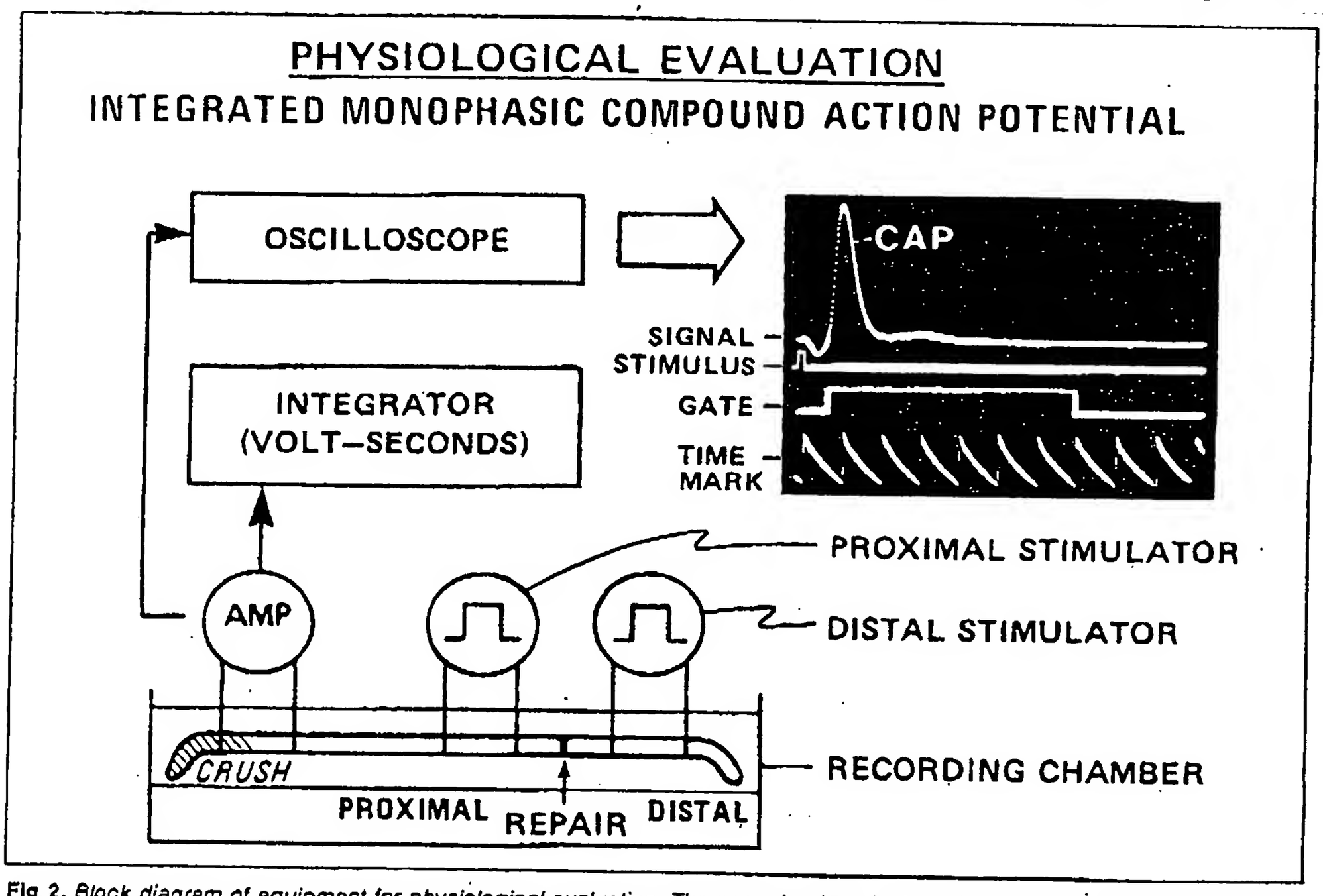

Fig 2. *Block diagram of equipment for physiological evaluation. The nerve is placed within the recording chamber across the distal and proximal pairs of stimulating electrodes and recording electrodes. The repair represents the site of severance, in the transected and repaired nerves, and the site of crush in the control nerves. The CAP from the proximal or distal stimulator is recorded by the proximal pair of recording electrodes (the nerve is crushed at its most proximal recording electrode to obtain a monophasic CAP). The recorded CAP is amplified, integrated (IMCAP) and also displayed by the oscilloscope. The CAP, the stimulus, the gate (time window of integration) and time mark references are photographed.*

The area recorded from stimulation of the *proximal* electrode measures the *total population* of *proximal* myelinated axons. By determining the area ratio (the area recorded from the distal stimulation divided by the area recorded from the proximal stimulation), we can obtain a measure of those myelinated proximal axons with distal connections normalized to the total proximal myelinated fiber population. This area ratio is expressed as a precentage. The limitations in this method are dealt with in detail elsewhere[84] but do not affect the overall conclusions of this report. The proportion of proximal axons with functional distal connections is only one of a number of factors which determine functional recovery.[1] We would expect that a nerve repair technique that results in an increased proportion of proximal axons with distal connections would result in an improved functional recovery. However, the magnitude of this effect, if any, remains to be determined in chronic experimental and clinical studies.

Histologic Evaluation

The nerves were removed from the recording chamber and fixed in 3% glutaraldehyde. To determine the architecture at the anastomosis, the repaired nerves were histologically evaluated by longitudinal sections stained with hematoxylin and eosin, and transverse sections stained with Bodian and Analine blue.

Results

Histologic Results Shortly After Repair

Comparisons of repairs by tubulization and suture were done on a total of six animals, one animal acutely at each of the following periods after repair: 2, 4, 6 and 8 weeks; two animals were studied acutely after 3 weeks. Grossly, the tubulization repair in this tensionless model held the nerve ends in accurate alignment and resulted in less surrounding fibrosis than the suture repairs.

Microscopically, the longitudinal and transverse sections at the anastomosis were evaluated for tissue reaction in the repaired nerves. The hypoantigenic collagen elicited a tissue reaction at the periphery of the nerve repair (Figs 3B, 3D). This tissue reaction showed no evidence of interfering with the regeneration within the fascicle in the suture repairs. The nylon sutures also elicited a tissue reaction (Figs 3A, 3C), which only interfered with regeneration if the suture was within the substance of the fascicle.

The anastomoses in three of the six animals were evaluated with transverse sections, one animal acutely at 3, 6 and 8 weeks. In the tubulization repairs, there was no evidence of regeneration being blocked by either the tube shrinking and constricting the nerve or by the tube collapsing and blocking the path of regenerating fibers. In the suture repairs, sutures placed in the perineurium did not interfere directly with regeneration, but sutures placed through the perincurium appeared to mechanically alter the course of regenerating fibers within the fascicle (Figs 3C, 3D).

Quantitative Physiological Evaluation of Uninjured Nerves

The area ratios for the 10 uninjured nerves had a mean of 97%, median of 97.5% and a range of 93 to 102%. We consider that these results substantiate that our entire experimental apparatus, including the nerve itself, is capable of sufficient accuracy, for the results reported here, since the changes observed after nerve repair are much larger than any that can be ascribed to our experimental apparatus or technique.

Quantitative Physiological Evaluation of Crushed Nerves

Since there is a marked clinical difference between the results of crush injuries and those involving transection of the nerve[1,2] in that crush injuries show a much greater return of function than do cuts, we did a study of three animals with a crush injury

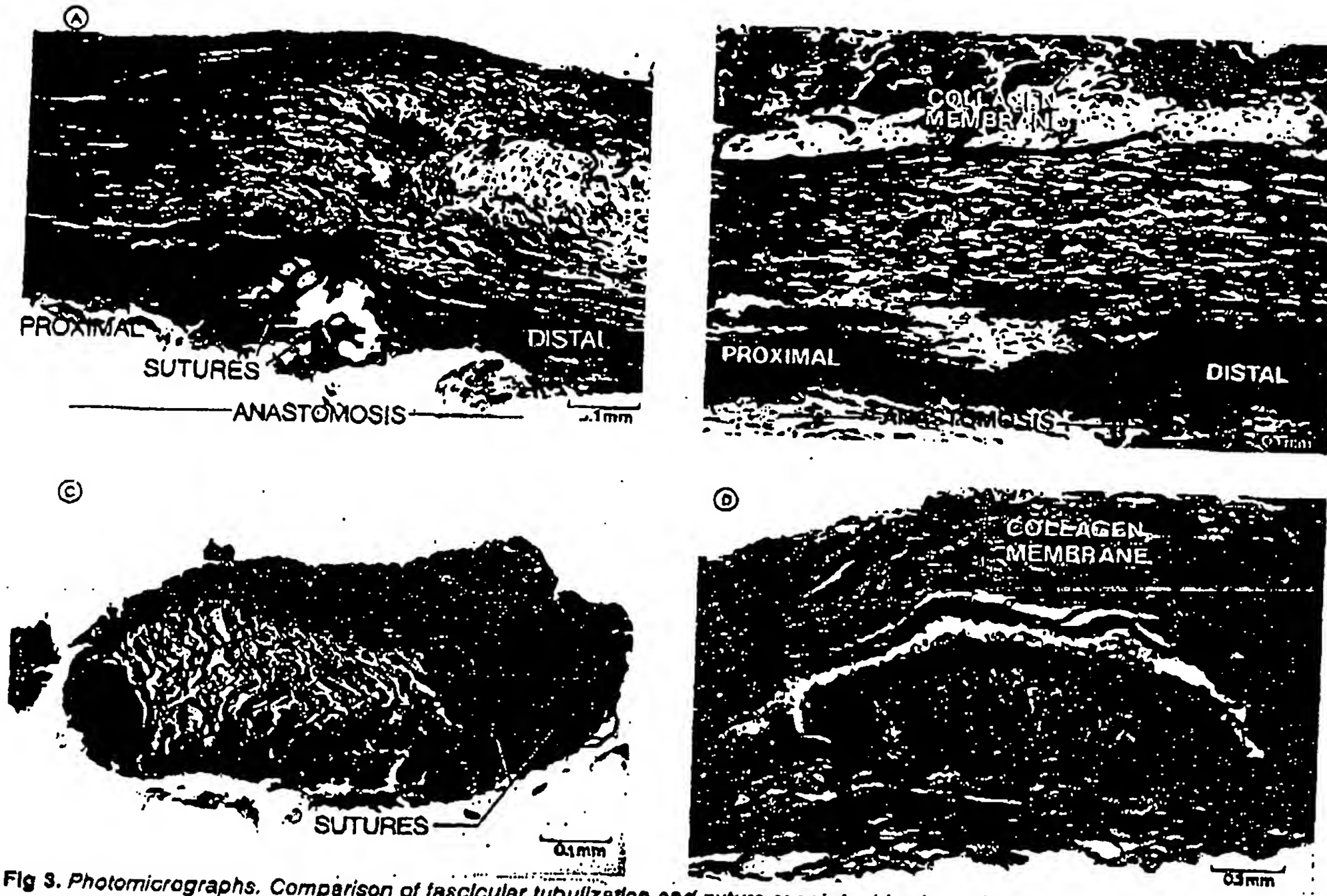

Fig 3. *Photomicrographs. Comparison of fascicular tubulization and suture repair by histology. (A, B) Comparison of representative longitudinal (H, E) sections at the anastomosis two weeks after transection and repair. (A) Perineurial suture repair. (B) Perineurial tubulization repair. (C, D) Comparison of representative transverse sections at the anastomosis three weeks after transection and repair (Bodian method). (C) Perineurial suture repair. (D) Perineurial tubulization repair.*

on one side and a sutured (transected and repaired) nerve on the other. Nerves on both sides had the same surgical exposure. The nerves from three rats were evaluated 10 months after the initial injury and surgery. There was a significant difference between the two types of injury ($p = .05$). The crushed nerves in every case showed greater regeneration than the sutured nerves. The area ratios of the crushed nerves (mean = 94%) were not significantly different than those ($p = 0.4$) of the uninjured nerves (mean = 97%) but were significantly greater than the area of the sutured nerves (mean = 61%) ($p = 0.05$). These results provide a control to indicate that surgical exposure of the nerve and local injury (crush) are not the cause of the lowered result seen in the sutured and fibrin blood clot repairs.

Quantitative Physiological Evaluation of Repaired Nerves

We have studied 10 animals in which the nerve was transected and repaired by tubulization on one side and suture on the opposite side. Physiological results for both 12 weeks and 24 weeks are presented in Table 1. In five of the animals the nerve of the tubulized repair was found to have elongated, curving back across the accompanying artery and vein. The results for these nerves did not appear to differ from the rest, and are included in Table 1. The area of the compound action potential recorded from the distal stimulator is ex-

Experimental Data From Fascicular Nerve Repairs
(Results: Area (IMCAP ratios expressed as %)

Time (weeks)	*Tube (matched)*	*Suture*	*Clot (independent)*
12	83*	46	
	84*	10	
	77	61	
24	74*	24	64
	86	44	31
	89	79	50
	88	45	59
	86	39	62
	83*	39	64
	70*	29	20

**Nerves which appeared to have elongated, having bowed out of the natural bed in the region of the repair.*

Table 1

pressed as a percentage of the area of the CAP recorded from the proximal stimulator. The area ratios indicate that in all 10 animals the nerve with the sutureless repair had a greater proportion of proximal myelinated axons that conduct across the anastomosis than did the *contralateral* sutured side (Table 1). This difference was statistically significant at 24 weeks ($p = .01$) and at 12 weeks ($p = .05$).

The fibrin blood clot fascicular nerve repairs were done on seven nerves. Quantitative population evaluation using area ratios demonstrated a mean of 50% for this group at 24 weeks. The fibrin blood clot results at 24 weeks were statistically inferior ($p < .001$) to the collagen tubulization results and were not statistically different than those of the suture repairs ($p = 0.38$) (Table 1).

Conclusions

A tube repair is essentially a fibrin clot repair with the addition of a hypoantigenic collagen membrane around the fascicle. A suture repair is essentially a fibrin clot repair with sutures through the fascicular perineurium. We conclude that the addition of a tube to the fibrin clot results in statistically superior regeneration compared to the fibrin blood clot alone at the fascicular level. We cannot, at this time, ascribe the superiority of our tubulization to the hypoantigenicity of the collagen membrane since we have not tried repairs with membranes of similar physical properties but greater antigenicity. We also conclude that sutures added to the fibrin clot repair do not statistically change the results of the fibrin clot repair.

The evaluation method used, the area ratio of the compound action potential, provides a measure of the entire connected proximal myelinated axon population which is not available with other evaluation methods.[34] The axonal population is only one of many factors which determines function, and its ability to predict clinical function has not been tested.

The primary objective for the surgeon is the reconnection of the severed ends of the nerve in a manner that will be most likely to achieve functional recovery. Fascicular perineurial tubulization might well improve the results of clinical nerve repair by increasing the numbers of axons which may have functionally useful distal connections, compared to perineurial suture and fibrin clot repairs. Although improved nerve regeneration has been demonstrated in our experimental model, its ultimate effect on clinical function has not been determined.

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